Arginine-Stimulated Hyperglucagonemia in Diabetic Pima Indians

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SUMMARY

Groups of 27 nondiabetic Pima Indians, 34 nondiabetic Caucasians, and 12 diabetic Pima Indians with recent onset of their disease received an arginine infusion to determine if (1) nondiabetic Pima Indians and Caucasians had a similar glucagon response to arginine and (2) diabetic Pimas had excessive glucagon response to arginine as reported in other racial groups. The fasting glucagon levels in the three groups were not significantly different. During arginine monochloride infusion (5 mg./kg./minute for 40 minutes) the diabetic Pimas had glucagon levels significantly higher at 10 minutes and at all sampling points thereafter than the normoglycemic Pimas. Plasma insulin levels also increased during the infusion but, notably, never differed significantly between these two groups. There was no significant difference in the glucagon levels at any sampling point between the nondiabetic Pimas and Caucasians. The differences in glucagon levels between the nondiabetic and diabetic Indians are similar to those differences reported between diabetic and nondiabetic subjects of other racial origins. DIABETES 25:404-07, May, 1976.

Glucagon levels in the fasting state and during intravenously administered arginine have previously been described in diabetics,[1-4] and although fasting glucagon levels in diabetics are generally similar to normals, mean glucagon responses to intravenous arginine are significantly greater in diabetics despite their hyperglycemia.

The Pima Indians of Arizona have the highest reported prevalence of diabetes in the world,[5] and those with diabetes have a similar frequency of the specific diabetic complications (e.g. retinopathy, nephropathy) as other races.[6,7] Except for the very high prevalence of diabetes, there appears to be no major difference in the clinical characteristics of idiopathic diabetes mellitus in this population from that of other races. Glucagon levels were measured in nondiabetic and diabetic Pima Indians and in nondiabetic Caucasians (1) to determine if the glucagon levels and responses seen in nondiabetic and diabetic Pima Indians were similar to or different from those of other racial groups and (2) to determine if Pima Indians have any abnormalities in plasma glucagon levels that might account for their propensity to develop diabetes.

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Accepted for publication January 13, 1976.

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MATERIALS AND METHODS

Subjects (Table 1)

Pima Indians aged 18 and over were recruited from among those individuals currently under continuing epidemiologic investigation.[5] Twenty-seven subjects (nondiabetic Pimas) were selected from those examined one or more times in the past 10 years and in these determinations had plasma glucose levels ≤140 mg./dl. two hours after a 75-gm. carbohydrate load‡ and whose parents had never had a plasma glucose >160 mg./dl. two hours after a similar carbohydrate load and were 45 years of age or older when last examined.

Twelve subjects with recent-onset diabetes (diabetic Pimas) were selected from those who had one or more plasma glucose levels ≤140 mg./dl. two hours after a 75-gm. carbohydrate load during the last five years and who had subsequently had plasma glucose levels >275 mg./dl. two hours after a 75-gm. carbohydrate load. Of these diabetics, one had been treated with insulin and six with oral hypoglycemics for up to 24 hours prior to this study. Five patients had previously received dietary treatment alone. A group of 34 Caucasian volunteers (nondiabetics) without a family history of diabetes were also examined.

Procedures

All subjects were instructed to take a high carbohydrate diet for three days prior to entering the hospital and were placed on a measured diet containing approximately 300 gm. of carbohydrate per day

‡Dexcola, Custom Laboratories, Baltimore, Maryland.

TABLE 1

General characteristics (mean ± 1 S.E.M.)

Group	No. of subjects	Sex	Age (years)	% Desirable weight
Nondiabetic Indian	27	11 M 16 F	25±1	142± 6
Diabetic Indian	12	4 M 8 F	34±2	166±13
Nondiabetic Caucasian	34	12 M 22 F	29±1	116± 4

for three days prior to study. To confirm the classification of subjects as nondiabetic or diabetic, they were given a standard oral glucose tolerance test (GTT) after an overnight fast using a 100-gm. carbohydrate load§ with plasma glucose determinations performed at fasting and one, two, and three hours. The nondiabetic subjects also received a 25-gm. intravenous glucose tolerance test (IVGTT), with frequent glucose determinations for one hour after a second 12-hour overnight fast.

An arginine infusion was then administered to all subjects after a subsequent 12-hour overnight fast. An indwelling venous catheter was placed in the arm and three baseline blood samples were obtained at 10-minute intervals. Through a second intravenous catheter in the opposite arm a 12.5 per cent solution of arginine monochloride (prepared by NIH pharmacy) was infused at a rate of 5 mg./kg./min. for 40 minutes. Blood samples were obtained at 5, 10, 20, 30, and 40 minutes after the start of the infusion. Those for glucagon and insulin determination (10 ml.) were collected in tubes containing 14 mg. of sodium EDTA and 5,000 units of Trasylol[8] in a volume of 0.5 ml., placed immediately in ice and centrifuged at 4° C. within one hour after collection. Plasma was separated, stored promptly at −20° C., and maintained in a frozen state until the time of assay in Dallas, not more than three months later. Samples (5 ml.) for plasma glucose determination obtained at the same times were placed immediately in tubes containing 30 mg. of sodium fluoride and were analyzed on the AutoAnalyzer by the ferricyanide method.[9] Glucagon levels were measured in duplicate by a modification of the previously described radioimmunoassay[10] using antiserum 30K. Insulin was determined by the Herbert modification of the Yalow and Berson radioimmunoassay.[11] All plasma samples were routinely examined for insulin antibodies by determining the per cent ^{125}I insulin bound to the subject's serum proteins in the absence of insulin antiserum. One diabetic who had received insulin for almost two years ending three months prior to the study was found to have insulin antibodies. Insulin levels were not determined in this subject.

Statistical analyses were performed by one-way analysis of variance followed by Duncan's multiple range test.[12] Covariance analysis was employed to make adjustments for differences in concomitant variables while investigating glucagon levels.

§Koladex, Custom Laboratories, Baltimore, Maryland

RESULTS

All nondiabetic Pimas and Caucasians had oral and intravenous GTTs, and in each the summation of the fasting, one-, two-, and three-hour plasma glucose levels during the oral GTT was ≤570 mg./dl.[13] and during the IVGTT the K-value was >1.00. On the oral GTT all the diabetics had two-hour plasma glucose levels >230 mg./dl. and all but two had fasting plasma glucose levels >135 mg./dl.

Table 2 and figure 1 show the glucagon levels of the three groups. Although the mean fasting glucagon levels (mean of three baseline determinations) in the diabetics (mean ±1 S.E.M.: 145±14 pg./ml.) and Caucasians (147±13 pg./ml.) were higher than in the nondiabetic Indians (118±11 pg./ml.), the differences were not statistically significant. This finding indicates that the fasting plasma glucagon levels were in the normal range in the fasting state in the diabetics in spite of considerably higher fasting glucose levels (vide infra). Following initiation of the arginine infusion, all groups had a brisk rise in glucagon. At five minutes the mean glucagon level was 302±29

TABLE 2

Glucagon response (pg./ml.) to arginine infusion in nondiabetic and diabetic Pima Indians and nondiabetic Caucasians (mean ±1 S.E.M.)

	Nondiabetic Caucasian (N=34)	Nondiabetic Pima (N=27)	Diabetic Pima (N=12)
Baseline	147±13*	118±11	145±14*
5 minutes	238±26	240±18	302±29
10 minutes	250±23	239±17	327±27†
20 minutes	266±22	271±18	362±29†
30 minutes	276±24	266±19	378±30‡
40 minutes	270±24	253±19	399±28§
Maximum increment	145±18*	175±15	271±22‡

*p>0.05 versus nondiabetic Pimas.
†p<0.01 versus nondiabetic Pimas.
‡p<0.005 versus nondiabetic Pimas.
§p<0.0005 versus nondiabetic Pimas.

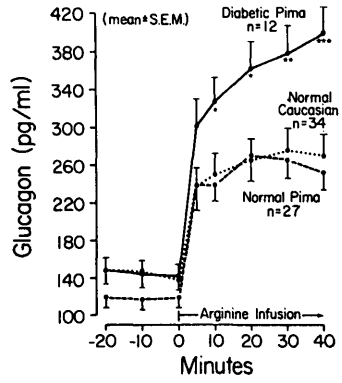

FIGURE 1

Plasma glucagon levels during arginine infusion in nondiabetic and diabetic Pima Indians and nondiabetic Caucasians (mean ± S.E.M.). *p<0.01 versus nondiabetic Pimas; **p <0.005 versus nondiabetic Pimas; ***p <0.0005 versus nondiabetic Pimas.

pg./ml. in the Pima diabetics, 240±18 pg./ml. in the nondiabetic Pimas, and 238±26 pg./ml. in the nondiabetic Caucasians. Glucagon levels in the three groups continued to rise, but at a greater rate in the diabetics. Mean glucagon levels were significantly higher in the diabetics than in either of the nondiabetic groups at 10 minutes and beyond ($p \leq 0.01$). The mean maximum increment in glucagon above baseline concentration was 271±22 pg./ml. in diabetics, as against 175±15 pg./ml. in the nondiabetic Pimas ($p < 0.005$) and 145±18 pg./ml. in the nondiabetic Caucasians ($p < 0.005$). The differences between the nondiabetic Indians and Caucasians were not significant at any time point.

Fasting and arginine-stimulated glucose and insulin responses in the Indians are shown in table 3. As expected, at all times the normal subjects' glucose levels were significantly lower than the diabetics'. The mean maximum increments in glucose, while greater in the diabetics, were not significantly different from the nondiabetic group. The mean insulin levels of the nondiabetic and diabetic Indians were similar, although there was greater variability in insulin levels in the diabetics. Insulin levels in the Caucasians[14] will be reported in detail elsewhere.

As noted above, glucagon levels—both fasting and during arginine stimulation—did not differ significantly among the nondiabetic Indians and Caucasians. When adjustments were made for differences in sex, ¶ age, obesity, and glucose and insulin levels by covariance analysis, the glucagon levels in the two groups still did not differ significantly either in the fasting state ($p > 0.17$) or at 40 minutes ($p > 0.08$).

¶While fasting glucagon levels were similar in both sexes, the 40-minute levels in the nondiabetic males and females were 332±30 pg./ml. and 220±14 pg./ml., respectively, indicating the importance of accounting for sex in such comparisons.

TABLE 3

Glucose and insulin response to arginine infusion in nondiabetic and diabetic Pima Indians (mean ±1 S.E.M.)

	Plasma glucose (mg./dl.)			Plasma insulin (μU./ml.)		
	Non-diabetic (N=27)	Diabetic (N=12)	p	Non-diabetic (N=27)	Diabetic (N=11)	p
Baseline	88±1	199±18	<0.0001	23±2	28± 5	NS
5 minutes	93±1	199±18	<0.0001	85±6	85±13	NS
10 minutes	96±2	203±17	<0.0001	60±5	63±10	NS
20 minutes	98±2	208±18	<0.0001	69±7	65±12	NS
30 minutes	98±2	211±16	<0.0001	62±6	67±16	NS
40 minutes	97±2	213±16	<0.0001	62±8	69±13	NS
Maximum increment	13±1	18± 3	N.S.	67±6	63±11	NS

Covariance analysis was also used to determine whether the significant differences in glucagon levels during arginine stimulation between the diabetic and nondiabetic Pimas could be explained by differences in age, sex, obesity, or insulin level. When these variables were held constant, however, there was still no significant difference in fasting glucagon levels between the two groups ($p > 0.09$), but a significant difference between the normals and diabetics remained during arginine stimulation (at 40 minutes; $p < 0.0005$).

DISCUSSION

This study confirms and extends previous reports that diabetes is associated with both a relative fasting hyperglucagonemia and an absolute hyperglucagonemia in response to arginine infusion.[1,3,4] The results demonstrate that the abnormal alpha-cell function reported in diabetes in other populations is also characteristic of the group with diabetes in the more genetically homogeneous and distinct racial group of Pima Indians.

In view of the extremely high prevalence of diabetes in the Pima Indians, the glucagon levels in Pima subjects who were the offspring of nondiabetic parents were examined to determine if abnormal levels in response to arginine stimulation were characteristic of Pima Indians in general or limited to those with diabetes. In fact, glucagon levels in the fasting state and during arginine infusion in the nondiabetic Pimas were found to be similar to those of nondiabetic Caucasians.

In response to the arginine infusion, insulin levels increased more than twofold in both the diabetic and nondiabetic Indian groups. On the other hand, the mean increments in plasma glucose levels (13

mg./100 ml. in the nondiabetic and 18 mg./100 ml. in the diabetic Indians) did not differ significantly despite the highly statistically significant greater mean increment in glucagon level in the diabetic group. These relationships are similar to those observed by others, and the low-order correlations between glucagon and glucose change[14] are not surprising in view of the many factors relating to glucoregulation.

The groups of subjects examined were not strictly comparable in terms of other characteristics, such as degree of obesity, mean age (table 1), and insulin levels. Thus, it was possible that these differences might be responsible for the differences in glucagon levels in the diabetic and nondiabetic Indians, as the nondiabetic Indians were younger and less obese than the diabetics. The nondiabetic Indians were also younger and more obese than the Caucasians, and this disparity might have obscured racial differences in glucagon levels between the Caucasians and Indians.

The relationship between glucagon and obesity has been controversial, with varying conclusions in the literature,[15-17] and the relationships between glucagon, sex, age, and insulin level have not been clearly delineated. After adjustment for the influence of obesity, age, sex, glucose, and insulin level, the similarity in glucagon responses during the arginine infusion in the nondiabetic Indians and Caucasians persisted, indicating that the equivalent glucagon responses were not explained by differences in these characteristics among the groups. After adjustment for the same factors (except glucose level), the highly significant difference remained between the diabetic and nondiabetic Pimas, indicating that the arginine-stimulated hyperglucagonemia of diabetes in the Pimas is unexplained by these factors and is substantially independent of them.

As the Pima Indian diabetics had both relative fasting and absolute hyperglucagonemia in response to arginine and as the fasting and arginine-stimulated insulin levels were similar in the diabetic and nondiabetic Indians, this study indicates that abnormalities in plasma glucagon levels are characteristic of diabetes at a stage when abnormalities in plasma insulin levels either in the fasting state or in response to an arginine infusion are not apparent.

ACKNOWLEDGMENTS

The authors wish to thank the Pima Indian volunteers of the Gila River Indian Community, whose cooperation and participation made this study possible. We also thank Ms. Janet Clark for secretarial assistance in the preparation of this manuscript, Ms. Virginia Harris for laboratory assistance, and Mr. Otto Morgenstern for computer-programming assistance.

This work was supported in part by contract HSM 73-73-165 and NIH grant AM 02700-16.

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